NIH-IRB Protocol #98-HG-0140:

A Phase I Study of Intralesional Administration of an Adenovirus Vector Expressing the HSV-1 Thymidine Kinase Gene (AdV.RSV-TK) in Combination with Escalating Doses of Ganciclovir in Patients with Cutaneous Metastatic Malignant Melanoma.

P.I. John C. Morris, M.D.

APPENDIX M-1

(1) SCIENTIFIC ABSTRACT

This phase I study will evaluate the toxicity and secondarily the response of patients with advanced malignant melanoma treated with a replication defective adenovirus expressing the *herpes simplex* virus type-1 thymidine kinase (HSV-*tk*) gene and escalating doses of the prodrug ganciclovir (GCV). HSV-*tk* when expressed in mammalian cells catalyzes the monophosphorylation of the non-toxic prodrug GCV by ATP. This is then further phosphorylated by cellular kinases, incorporated into DNA, where it inhibits DNA synthesis and ultimately results in cell death. HSV-*tk*/GCV "suicide gene" therapy is undergoing evaluation as an anticancer treatment in a number of Phase I-III trials. An early meta-analysis of 103 patients enrolled in various HSV-*tk*/GCV cancer gene therapy trials in which information was available, reported major tumor regressions in 8 of 62 (12.9%) evaluable patients.[1] To date, the results of only two trials have been reported in their entirety: Ram and colleagues at the NIH attempted *in situ* transduction of tumors with a recombinant HSV-*tk* expressing retrovirus by inoculating murine vector producer cells directly into the brain tumors of 15 patients.[2] They subsequently administered GCV 5 mg/kg I.V. twice daily for 14 days. A response was defined as a $\geq$ 50% decrease in tumor volume on a contrast enhanced MRI-scan. These workers were able to show successful gene transduction in two biopsied lesions and 5/19 treated lesions responded to therapy. The median duration of response, however, was short at 7.8 weeks. In a phase I trial conducted at the University of Pennsylvania, 21 patients with malignant pleural mesothelioma were treated in dose-escalation cohorts with an HSV-*tk* expressing adenoviral vector followed by GCV 5 mg/kg I.V. twice daily for 14 days.[3] Maximum tolerated dose of the vector was not reached in this study and successful gene transfer was demonstrated in 11 of 20 patients. No definitive evidence of tumor regression was noted despite administered doses of vector up to 1×10^{12} PFU.

Work in the laboratories of the Clinical Gene Therapy Branch, NHGRI and by others have indicated a steep GCV dose-response effect in the HSV-*tk* system. HSV-*tk* expressing tumor cells are able to generate higher intracellular concentrations of GCV-triphosphate, the active form of GCV when exposed to increasing concentrations of GCV in the media. GCV has been shown to exhibit an dose-response effect both *in vitro* and in animal tumor models. Furthermore, studies have shown that the GCV blood levels achieved with the doses used in the preclinical mouse models are substantially higher than that seen in patients using standard clinical doses (5 mg/kg twice daily). We propose that the low rate of responses reported to date in HSV-*tk*/GCV cancer gene therapy trials may in part be a result of inadequate doses used of the prodrug ganciclovir.

This study will attempt to address the ganciclovir dose-response effect in an adenovirus mediated HSV-*tk* gene therapy trial in patients with advanced melanoma. Adult patients with metastatic (stage IV) malignant melanoma who are not curable by currently available treatments and who have at least one accessible, discreet, cutaneous or subcutaneous lesion of ≤ 3 cm^3 volume will receive intratumoral injections of a genetically engineered adenovirus vector (AdV.RSV-TK) expressing the HSV-*tk* gene at a dose of 1 x 10^{10} plaque forming units (P.F.U.)/cm^3. Forty-eight (48) hours after administration of the vector, patients will receive intravenous GCV every twelve (12) hours for seven (7) days. This study will involve the escalation of the total dose of GCV administered patients from 5 mg/kg to 20 mg/kg q12 hours in patient cohorts of 2.5 mg/kg. Patients will be closely monitored for local and systemic toxicity. Patients will also be evaluated for response of their melanoma (both the treated lesion and other sites of untreated disease). Patients will undergo blood sampling to determine the pharmacokinetics of high dose GCV and the effect of this treatment on serum cytokine levels at various time points during the treatment. The goal of this study is to determine the maximum tolerated dose (MTD) of GCV which can be administered in combination with 1 x 10^{10} P.F.U./cm^3 of AdV.RSV-TK, and the dose limiting toxicity (DLT) of HSV-*tk* gene therapy in combination with high dose GCV in a cutaneous tumor model before proceeding to clinical trials involving visceral tumors. A secondary goal will be to determine the response rate of the treated lesions and possible immunological 'bystander" effects on remote sites of untreated tumor.

References:

1. Roth J.A. and Cristiano, R.J.: Gene therapy for cancer: What have we done and where are we going? *J. Natl. Cancer Inst.* 89: 21-39, 1997.

2. Ram, Z., Culver, K.W., Oshiro, E.M., et al: Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nature Med.* 12: 1353-1361. 1997.

3. Sterman, D.H., Treat, J., Litzky, L.A., et al. Adenovirus mediated Herpes simplex virus thymidine kinase/ganciclovir gene therapy in patients with localized malignancy: Results of a phase I clinical trial in malignant mesothelioma. *Hum. Gene Ther.* 9: 1083-92. 1998.